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**Board Number: B239**

**Imaging of three-dimensional single molecule dynamics with cellular context: Antibody trafficking and interaction with cell membrane and sorting endosomes.**

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Prostate-specific membrane antigen (PSMA) is an important biomarker for prostate cancer cells. As such, it is a common target for antibody-based therapies. For example, of particular interest is the use of PSMA-specific antibodies that are conjugated with a toxin that kills prostate cancer cells. We analyze here the pathways of PSMA-specific antibodies, from prior to their first binding to PSMA at the plasma membrane to their arrival at, and continued moment in, sorting endosomes. To investigate the trafficking of these antibodies in detail, we developed a novel multi-dimensional microscopy imaging modality called remote focusing-multifocal plane microscopy (rMUM). rMUM importantly enables the visualization of not only the 3D trajectories of the PSMA-specific antibodies at the single molecule level, but also the cellular structures with which the antibodies interact during their trafficking. Specifically, the rapid movement of the antibodies in 3D space is captured using multifocal plane microscopy (MUM), a modality that simultaneously images distinct focal planes within the cell sample. At the same time, the surrounding cellular structures are captured in the form of z-stack images using the technique of remote focusing. By making possible the observation of single molecule dynamics within the relevant cellular context, rMUM allows, in our current application, the identification, and analysis of different stages of the PSMA-specific antibody trafficking pathway. These stages can be summarized as follows. First, quantum dot-labeled PSMA-specific antibody is observed in the cell exterior to very rapidly diffuse towards the plasma membrane. Following a period of both unconstrained and constrained diffusion on the lateral plasma membrane, the antibody undergoes endocytosis and is immediately transported towards a sorting endosome by highly directed motion. Subsequently, the antibody is observed to enter the sorting endosome and then diffuse along its spherical surface on the endosome's inner leaflet. The results obtained thus provide a significantly more complete understanding of PSMA dynamics at the single molecule level and within the cellular environment.

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**Visualizing enzymatic and cellular activities during tissue morphogenesis using ex vivo two-photon FRET microscopy.**

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Morphogenesis is a critical step in embryogenesis during which tissue/organ transforms into its functional shape. The drastic change in tissue morphology signifies active cellular reorganization and extra-cellular matrix remodeling. Enzymatic activity vital to this process, especially that of matrix metalloproteinase (MMP), attracts a lot of research interests but proves difficult to investigate using traditional methods. Förster resonance energy transfer (FRET) has emerged as a powerful technique for

investigating biochemical interaction, but its usefulness in live tissues has not been well established. Here we use a genetically encoded FRET biosensor with ex vivo two-photon microscopy to demonstrate a clear increase in MT1-MMP activity at the tip of a growing feather bud, which coincides with increased cellular motion, sometimes across the epidermal-dermal border, and weakened laminin structure in the basement membrane. Laminin and collagen III are newly synthesized at the tip, strengthening the newly formed structure. These events also exhibit spatio-temporal correlation with increased Src activity observed using another genetically encoded FRET biosensor. Our ex vivo biochemistry approach provides insights into the spatial and temporal profile of enzyme activities, and together with traditional approaches, offers a comprehensive understanding of the morphogenetic process. Furthermore, it can readily lend itself to the studying of other biological processes, such as cancer metastasis, where enzymatic activity plays a central role.

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**Board Number: B241**

### Targeting Exogenous $\beta$ -Defensin to the Endolysosomal Compartment via a Biotic Guided Missile System.

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Viral, bacterial and protozoal infections are a preoccupation with respect to the evolution of virulence and iatrogenic enhancement due to cross-species transmission and antibiotic resistance. A number of bacteria, viruses and protozoa take advantage of receptor-mediated endocytosis, a critical function of eukaryotes, by binding to surface proteins and receptors and by using their endocytic pathway to infect cells. Once in the endosomes and/or phagosomes, the pathogens complete their life cycle by overriding normal lysosomal functions. Most notably, pathogens evade lysosomal degradation by the selective retention of Rab GTPases in the membranes of the vacuoles that they occupy in host cells during infection, inhibiting the fusogenic function and acidification of these organelles and/or the activity of the lysosomal hydrolases. Recently, our laboratory identified the lysosomal targeting signal of prosaposin, which is recognized by the sorting receptor "sortilin". Based on this evidence, we tested whether the antimicrobial peptide  $\beta$ -Defensin linked to the targeting sequence of prosaposin ( $\beta$ -Defensin-prosaposin-V5) could be redirected from its secretory pathway to the endolysosomal compartment. To this end,  $\beta$ -Defensin-prosaposin-V5 was transfected into COS-7 cells. The subcellular distribution of  $\beta$ -Defensin-prosaposin-V5 was analyzed by confocal microscopy and differential centrifugation. Confocal microscopy demonstrated that  $\beta$ -Defensin-prosaposin-V5 overlaid with the lysosomal marker LAMP1, indicating that the construct reached endosomes and lysosomes. Differential centrifugation also showed that  $\beta$ -Defensin-prosaposin-V5 was in the lysosomal fractions. In addition, a binding inhibition assay demonstrated that  $\beta$ -Defensin-prosaposin-V5 bound specifically to sortilin. Similarly, the delivery of  $\beta$ -Defensin-prosaposin-V5 was abolished after overexpressing a truncated sortilin. These results indicate that the prosaposin C-terminus and D/C-domain (prosaposin targeting sequence) was an effective biotic "guidance system" to redirect  $\beta$ -Defensin to the endolysosomal compartment. In the future, this and other fusion proteins with antimicrobial properties will be assembled to our "biotic missile" to target pathogens growing within these compartments. Supported by NSERC Canada.